KILLING, STAINING AND MOUNTING PARASITIC NEMATODES*

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In some work on *Heterakis papillosa* of the domestic fowl I found that the ease with which fluids will penetrate the cuticula depends much on the method by which the worms have been killed.

Parasitologists are mostly following Looss in killing nematodes by means of hot solutions, usually of alcohol. This has the advantage of straightening the worms out as they die. The straightening seems to be due to the heat, as hot water will have the same effect. Cold concentrated killing solutions will usually cause the worms to die in very much contorted shapes.

When nematodes are killed by either of the above methods staining is rendered very difficult and mounting in balsam usually is impossible unless the cuticula is broken in one or more places. The final mounts are usually of a very inferior nature even tho all possible precautions are taken in changing from one fluid to another.

This resistance to penetration is not a normal function of the nematode cuticula as in the living worm fluids seem to penetrate the cuticula very readily. The resistance is due to a change in the character of the cuticula. This change is also indicated by the curling up or rolling up of free parts of cuticula such as wings or a pointed tail. These parts behave as tho the cuticula becomes semifluid during the killing process and then sets in an impervious condition.

This change can be prevented to a great extent by employing the method used by Dr. Cobb in killing free living nematodes. The essential point seems to be to start with a very weak solution. The nature of the chemical substance used is undoubtedly also of great importance. I have obtained very good results only with ethyl alcohol. Methyl alcohol and a solution of mercuric chloride both gave very inferior results; but this may have been due to the use of too strong solutions. Not only does this method prevent the change in the cuticula, but it also usually gives just as straight specimens as those killed in hot solutions.

In my work I still find it more convenient to use the string siphon differentiator as first described by Magath (Trans. Amer. Micr. Soc., 35: 245-256) and with the modifications described by me (Ill. Biol. Mon-

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ogr., Vol. 5, No. 2, p. 13) than to use Dr. Cobb's apparatus. An attempt to use his differentiator almost invariably results in the loss of valuable material because it cannot be found after the process has been completed. If the material is abundant so that the loss of a few specimens is of no consequence his method has the advantage of using less fluid and occupying less space. I find difficulty also in regulating the flow of liquid in his differentiator as that depends not only on the size of the capillary outlet but also on the weight of the column of liquid and the concentration of the alcohol, and the capillary tube may become clogged at any time.

In the string siphon method the specimens may be placed in a dish the bottom of which can be examined everywhere by means of a magnifying glass, binoculars or even a very low power of a compound microscope. The specimens need not be disturbed during the entire process of dehydration and clearing. By the method I am now using a single nematode of microscopic dimensions may be placed in a deep embryological watch glass while alive, and killed, stained, dehydrated, cleared and infiltrated with balsam without being disturbed. The only transfer necessary is that from the watch glass to the final mount on the slide.

By this method the specimens are placed in a watch glass or small stender dish in eight to nine tenths per cent salt solution. By means of a string siphon an amount of 5-7 per cent ethyl alcohol equal to four or five times the volume of salt solution is passed over the specimens. This is followed by an equal amount of 10 or 15 per cent alcohol and so on. Each time the concentration of the alcohol is increased by 5 to 10 per cent. The size of the string should be so regulated that it requires from 6 to 12 hours for one change of fluid to take place.

The staining may be done anywhere in the course of the dehydration. I usually stain with Delafield's or Boehmer's hematoxylin in 70 per cent alcohol with the addition of a small amount of acetic acid to aid penetration and to avoid the necessity of destaining. This is followed by a weak solution of sodium or potassium acetate in 75 or 80 per cent alcohol. The dehydration is then continued.

Absolute alcohol is followed by 1-3 xylene in alcohol, 2-3 xylene in alcohol and pure xylene. If specimens are to be sectioned they are now imbedded in paraffin by the method described in my paper quoted above. If they are to be studied as cleared specimens or mounted in balsam they are next passed from xylene to synthetic oil of wintergreen just as they were passed from alcohol to xylene. This gives the specimens a maximum amount of clearing and makes excellent preparations for study at that time. Mounting in balsam renders the specimens more opaque and makes them appear more like they did in xylene.

To mount in balsam it is usually best to infiltrate the specimens first by removing all but a thin layer of the oil and then adding a small

lump of dry Canada balsam every day or so until the solution has acquired about the consistency of the balsam to be used for the mount. For permanent mounts it is best to use balsam dissolved in xylene even tho this does not retain the transparency of the object because balsam in oil of wintergreen requires months to harden. Gum dammar may be used for mounting but not for the process of infiltration as it does not dissolve completely in the methyl salicylate.